In all living systems carotenoids are the most widespread pigments with important structural and functional roles. These pigments can be essential for maintaining the membrane integrity, but they might also contribute to the regulation of membrane fluidity. In photosynthetic organisms carotenoids can function as accessory lightharvesting pigments, but they also serve as photoprotective agents, especially when the organisms are exposed to excess light. In particular, carotenoids are able to quench triplet excited states of chlorophylls, and directly scavenge singlet oxygen. Beside the protective functions, carotenoids also possess structural roles in some protein complexes, such as photosystem I (PSI) and Photosystem II (PSII). We summarized our knowledge in a review article Domonkos et al. (2013)

In the present project, we investigated the consequences of altered carotenoid composition for the structure, function and supramolecular organization of photosynthetic complexes (e.g. PSI, PSII and phycobilisomes) in cyanobacterial model systems. Cyanobacteria are prokaryotic photosynthetic organisms, the ancestors of plant chloroplasts. They were fundamental participants in the formation of the oxygenic atmosphere on Earth. Nowadays cyanobacteria represent an ecologically important group especially in the oceans; they have a major role in carbon- and nitrogenfixation and are often present as symbiotic partners. In cyanobacteria the most abundant carotenoids are β-carotene and various xanthophylls, such as synechoxanthin, canthaxanthin, caloxanthin, echinenone, myxoxanthophyll, nostoxanthin and zeaxanthin.

A. The role of carotenoids in the structure and function of photosynthetic complexes

We studied several *Synechocystis* mutants impaired at various steps of carotenoid biosynthetic pathway and characterized them using picosecond time resolved low temperature (77K) fluorescence spectroscopy, Circular-Dichroism (CD) spectroscopy combined with biochemical methods, and electron microscopy. The xanthophyll deficient crtR/O mutant can grow photoautotrophically while the completely carotenoid-less ΔcrtB having extreme light sensitivity and is only capable of growing heterotrophically in the dark. We also studied the crtH mutant either cultivated under photoautotrophic or heterotrophic growth conditions. In order to distinguish the carotenoid induced changes from the ones induced by the growth conditions, the wild type cells were grown under photoautotrophic and heterotrophic (dark) growth conditions, as well.

The pigment composition of mutants used in this study was determined by HPLC. The carotenoid composition of the photoautotrophic and heterotrophic WT cells does not differ significantly. The xanthophylldeficient crtR/O cells contain no zeaxanthin, echinenone, but have deoxy-myxoxanthophyll instead of the myxoxanthophyll. In the photoautotrophic crtH mutant lacking the pro-lycopene isomerase, a large amount of cis-carotene is present under both growth conditions indicating that the isomerization of the cis-carotene is the rate-limiting step of the synthesis. In addition, all carotenoid classes are present but their relative amounts are different than in the WT. In crtH cells grown in the dark heterotrophycally, no β-carotenes or xanthophylls are present. The carotenoid deficientΔcrtB cells contain only chlorophyll and a small amount of unknown non-carotenoid derivatives.

A1. Influence of carotenoids on photosystem I structure

In cyanobacteria, especially when grown under low-light intensity, most PSI is found in trimeric form. The crystal structure of PSI trimer from T. elongatus has revealed the presence of 22 β-carotenemolecules per monomer. In the present project using non-denauring and 2D gelelectrophoresis methods we demonstrated that the carotenoid-deficient ΔcrtB mutant contains predominantly PSI monomers and only a few PSI trimers. Despite the relative abundance of carotenoids in PSI, the basic function of PSI is only slightly affected in the carotenoid-deficient mutant. The *in vivo* decrease of PSI trimers as compared to the monomers was confirmed using picosecond fluorescence measurements. Since PSI trimers, in general, contain more long-wavelength Chlorophylls (LWCs) than PSI monomers, the substantial decrease of the LWCs in the PSI fluorescence signal of carotenoid-deficient cells, also indicates a considerable decrease in the trimer/monomer ratio as compared to WT cells.

Previously, xanthophyll molecules have also been observed in PSI preparations that might be explained by co-purification of xanthophylls, or by assuming that PSI trimers contain loosely connected xanthophylls, which are lost upon crystallization. We also recorded the presence of echinenone and zeaxanthin in the isolated PSI trimers. These two xanthophyll species are among the most abundant xanthophylls in this cyanobacterial species. They could be part of the PSI complex or be embedded in the membrane in the vicinity of PSI. Remarkably, in xanthophyll-deficient (crtR/O) cells protein analysis also showed slightly less PSI trimers and relatively more PSI monomers than in WT cells, which is accompanied by a decrease of LWC contribution to the fluorescence. Comparing the circular-dichroism (CD) spectra of WT and ΔPsaL mutant containing only PSI monomers, we could identify the *in vivo* CD fingerprint of PSI trimer. The decreased CD signal at around 515 nm also indicates the decreased PSI trimer to monomer ratio in the xanthophyll-deficient (crtR/O) mutant *in vivo*.

In our experiments, the changes of the PSI related CD signal does not essentially coincide with alteration of the LWC fluorescence of the PSI. Due to the contradictory behaviour of the PSI attributed CD and fluorescence signal in the absence of either zeaxanthin or echinenone, we suppose that these xanthophyll species influence the structure of PSI, specifically, in a different manner in vivo.

Our fluorescence spectroscopic results revealed specific structural changes manifested in altered pigment-pigment or pigment-protein interactions within PSI complex in the absence of zeaxanthin and echinenone. These structural modifications of the complexes seem to destabilize the PSI trimeric complexes and eventually result in an increased propensity for monomerization. Although zeaxanthin is present in a much lower level in the isolated complex, it is structurally more important than echinenone for maintaining the trimeric PSI complex.

A2. Influence of carotenoids on photosystem II structure

Although PSII contains less carotenoids than PSI (12 vs. 22 β-carotenes per monomer, in T. elongatus), carotenoids are essential for the assembly of PSII dimers in cyanobacteria. Accordingly, in carotenoid-less *Synechocystis* cells only trace amounts of the partially assembled PSII subcomplex can be detected, as was demonstrated earlier. We also could not distinguish a clear PSII fluorescence signal from the carotenoid-deficient cells. Our results show that the production of carotenoids by photoisomerization only, without the CrtH-

catalyzed pathway, results in partially impaired PSII functioning. The relatively fast fluorescence decay observed in these cells, as compared to ΔcrtB cells, indicates a considerable amount of functional PSII, which is capable of photochemical quenching. However, the amount of active PSII complexes seems to be lower than in WT cells.

Our protein analyses obtained for xanthophyll deficient mutants revealed a significant decrease in the amount of detected PSII dimers in low-light grown cells, but no corresponding change was seen in the PSII related *in vivo* fluorescence indicating that PSII is probably less stable and disassembles in the PAGE. These observations support the notion that the assembly of functional PSII requires the presence of β- carotene, whereas xanthophylls seem to have a minor, stabilizing function even under low-light conditions.

A3.The role of carotenoids in the structure and function of phycobilisomes

In cyanobacteria, peripheral antenna complexes, the phycobilisomes (PBSs), serve as light-harvesting antennae for the photosynthetic complexes. In PBSs the phycobilin pigments (phycocyanobilin, phycourobilin, phycoerythrobilin, phycobiliviolin) attached to phycobiliproteins (phycocyanin, allophycocyanin, phycoerythrin, phycoerythrocyanin) are responsible for light harvesting. In Synechocystis each PBS contains approximately six phycocyanin (PC) rods attached to the three allophycocyanin (APC) core cylinders. Each PC rod comprises typically three hexameric disks while all the APC core cylinders consist of four trimeric disks. There are various linker proteins, which are responsible for maintaining the PBS structure.

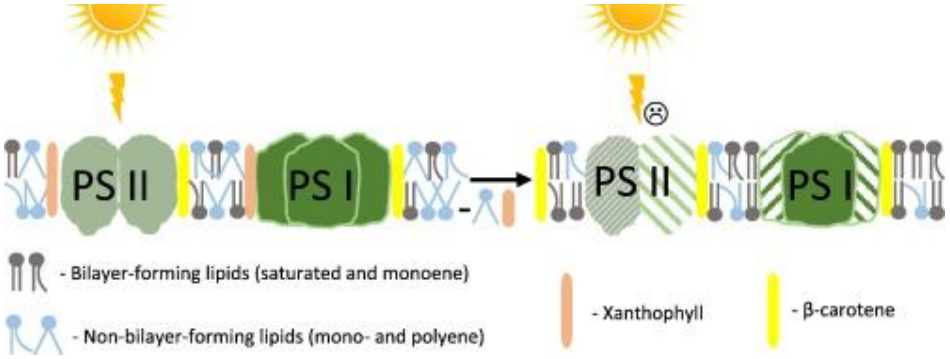
Although there is no report on the presence of carotenoids in PBSs, we have found that they strongly influence PBS integrity. In carotenoid-deficient (ΔcrtB) cells time-resolved fluorescence at room temperature revealed a high level of energetically disconnected, non-transferring PC units, which are not present in WT cells. Further measurements on this Car-less mutant showed the presence of assembled PBSs as well, but with reduced size. In the mutant PBSs we detected faster excited-state equilibration with the cores using time-resolved fluorescence which is attributed to the reduced length of radial rods, a notion confirmed by their protein composition . Our results imply that carotenoid-deficient PBSs contain predominantly rods with only one or two hexameric PC units, although small amounts of full-length rods, composed of three hexameric units, are also present. Besides the fully assembled PBSs, two fractions of phycobiliprotein complexes were separated by sucrose density gradient in the Carotenoid-less mutant. Both fractions show the typical PC fluorescence, but they differ in size. We conclude that in Carotenoid-deficient cells most of the PBSs possess a reduced number of the peripheral PC rods, and that part of the PC is present as unconnected units. It should be noted that the xanthophyll-less crtR/O mutant contains properly assembled PBSs, similar to WT cells. Therefore, we concluded that the lack of β-carotene may cause PBS distortion. Assuming a direct PBS-stabilizing role for carotenoids would imply the presence of a carotenoid molecule inside or in the vicinity of the PBS rods, but up to now there is no evidence supporting this assumption. Therefore, an indirect effect of the carotenoid composition on the structure of the PBSs seems more likely. Our results revealed a decrease of the rod linker

proteins at cellular level. We are tending to conclude that PBS degradation occurs due to the enhanced degradation under β-carotene limitation. We used nitrogen starvation to enhance the enzymatic PBS degradation and found a delayed degradation of the PC units. Our results suggest that an insufficient degradation rate of phycobiliproteins and/or an increased disassembly of PBS can be accountable for the increased amount of unconnected PC units upon limited β-carotene availability.

B.The role of carotenoids in the oligomerisation state of PSI

PSI trimer to monomer ratio in intact cyanobacterial cells and isolated thylakoids was analysed by two noninvasive, *invivo* methods; low-temperature fluorescence emission and circular dichroism spectroscopy. We measured fluorescenceemission spectra of cells upon chlorophyll (Chl, 436 nm) excitation. All three species – *Synechocystis* sp. PCC 6803, *Anabaena* sp. PCC 7120, and *Spirulina platensis* – showed shifted Chl peak, indicating they have different spectralproperties. CD spectroscopy revealed the highest intensity at 515 nm (PSI peak) in *Spirulina platensis* cells, which mayoriginate from PSI multi-oligomerisation. The most sensitive response to heat treatment in this strain was theoligomerisation of PSI RCs. PSI dimers and tetramers in *Anabaena* cells showed smaller changes of the CD signal uponthe heat treatment compared to that of *Synechocystis* WT. The lack of γ-linolenic acid affected the filament morphology by the loss of the spiral shape and the PSI monomerisa at al.(2018).

B. Polyunsaturated lipids are important components of photosynthetic membranes. Xanthophylls are the mainphotoprotective agents, can assist in protection against light stress, and are crucial in the recovery from photoinhibition. In order to study the little-known cooperative effects of lipids and carotenoids. Electron microscopic investigations confirmed that in the absence of xanthophylls the S-layer of the cellular envelope is missing. In wild-type (WT) cells, as well as the xanthophyll-less (RO), polyunsaturated lipid-less (AD), and the newly constructed ROAD mutants the lipid and Car compositions were determined by MS and HPLC, respectively. We found that, relative to the WT, the lipid composition of the mutants was remodeled and the Car content changed accordingly. In the mutants the ratio of non-bilayer-forming (NBL) to bilayer-forming (BL) lipids was found considerably lower. Xanthophyll to β-carotene ratio increased in the ADmutant. In vitro and in vivo methods demonstrated that saturated, monounsaturated lipids and xanthophylls may stabilize the trimerization of PhotosystemI. Fluorescence induction and oxygen-evolving activity measurements revealed increased light sensitivity of RO cells compared to those of the WT. ROAD showed a robust increase in light susceptibility and reduced recovery capability, especially at moderate low and moderate high temperatures, indicating a cooperative effect of xanthophylls and polyunsaturated lipids. We suggest that both lipid unsaturation and xanthophylls are required for providing the proper structure and functioning of the membrane environment that protects against light and temperature stress (Zakar T et al., 2017).



The figure shows how lipids and carotenoids affect the membrane structure anmd photosynthetic funtions.

We were able to demonstrate cooperative effect of carotenoids, lipids and proteins in construction of photosinthetic complexes and in their functions.